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TITLE: Quantitative PET Imaging with Novel HER3-Targeted Peptides Selected by Phage Display to Predict Androgen-Independent Prostate Cancer Progression

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14. ABSTRACT
Funding from this award has permitted the development of a highly specific peptide that targets HER3 for prostate cancer imaging. The peptide was labeled with a PET imaging radionuclide and injected into mice bearing human prostate cancer. The peptide accumulated at high levels in the tumors, and excisional analysis revealed quantitative accumulation of the peptide in tumors that was linearly correlated with HER3 levels. Biodistribution analysis revealed low off-target accumulation and rapid clearance through the renal system, consistent with small peptides. The peptide represents a promising clinical lead for HER3 imaging in patients with castration resistant prostate cancer.

15. SUBJECT TERMS
Castration Resistant Prostate Cancer, HER3, PET Imaging, Phage Display

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1. Introduction

The subject of this research is the design and testing of a PET imaging agent for the detection and characterization of castration resistant prostate cancer. It has recently been demonstrated that human epidermal growth factor-3 (HER3) is implicated in the transition from castration sensitive to castration resistant prostate cancer. As such, the purpose of this research is to select by phage display a suitable PET imaging agent for *in vivo* quantification and subsequently characterization of castration resistant prostate cancer. The selected peptide will be radiolabeled, injected into mice bearing prostate cancer and imaged.

2. Keywords

Castration Resistant Prostate Cancer, HER3, PET, Phage Display

3. Accomplishments

Major Goals (Research Specific Tasks)

Research-Specific Tasks:

Specific Aim 1: Optimize and Characterize HER3 Targeted Peptide		
Subtask 1: Utilize phage display-mediated shotgun alanine scanning to isolate variants of the selected HER3 peptide that bind with higher affinity and specificity than the first-generation peptide.	1-9	Dr. Larimer
Subtask 2: Test three independent scaffolds, including PEG, amino acid and branched peptide constructs for improved HER3 targeting through a multivalent increase in peptide avidity.	6-12	Dr. Larimer
Subtask 3: Measure biodistribution and PET imaging uptake of the parent, affinity maturated and highest affinity scaffold in mice bearing HER3-expressing PC3 xenografts. Cell lines used: PC-3/ATCC (Internal Lab Stock)	6-15	Dr. Larimer
Milestone(s) Achieved: Identification of a peptide sequence and configuration that binds with <100 nM affinity and specific HER3 targeting in vivo	12-14	Dr. Larimer
Specific Aim 2: Determine the changes in radiolabeled HER3 peptide tumor uptake during androgen withdrawal therapy.	15-24	Dr. Larimer
Subtask 1: Perform PET imaging in mice bearing androgen sensitive resistant human prostate cancer xenografts throughout androgen withdrawal until tumor progression. Cell lines used: PC-3, MDA-PC-2b/ATCC (Internal Lab	15-22	Dr. Larimer

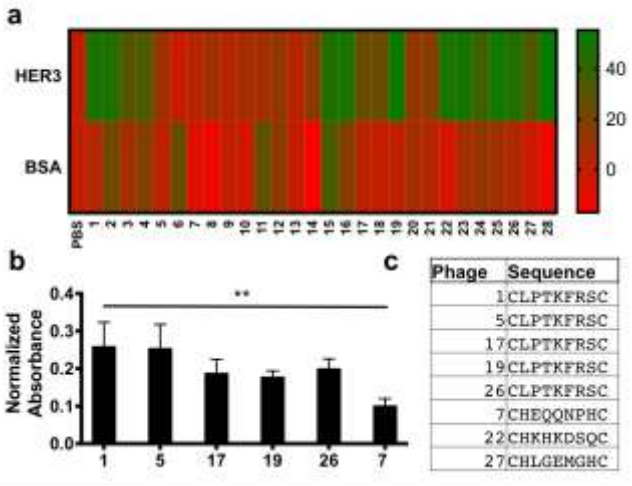
Stock)		
Subtask 2: Correlate changes in peptide uptake with protein expression and cell signaling changes *ex vivo*. Cell lines used: PC-3, MDA-PC-2b (Internal Lab Stock)	18-24	Dr. Larimer
Milestone(s) Achieved: Positive prediction of castration resistant prostate cancer progression with supporting ex vivo	22-24	Dr. Larimer

1) Major Activities: The major activities included the development of a second-generation peptide by phage display that bound with higher affinity than the first-generation peptide. This peptide was tested both *in vitro* and *in vivo* and demonstrated to bind specifically and quantitatively to HER3.

In the second year of funding, we have gotten quotes for synthesis of the branched peptide and have also devised a strategy for testing a longer peptide scaffold for improved biodistribution. We have also begun synthesis of the peptide developed in this proposal for clinical translation, and have been working to develop IND-enabling studies for clinical translation. Finally, we have converted the initial patent application to a PCT in anticipation of complete filing.

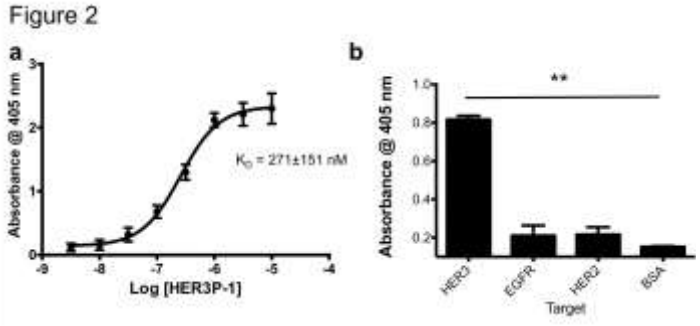
2) Specific Objectives: The first specific objective was to develop a targeted peptide for HER3 than the original peptide. This was accomplished using a phage display selection in which 28 new peptide sequences were screened (Figure 1A). Interestingly, the selection converged upon a single HER3 specific sequence CLPTKFRSC. This sequence was shown to be highly specific and conserved in HER3-avid phage, but not in non-specific phage isolated from the same selection. As such this peptide was pursued for further *in vitro* analysis.

Figure 1



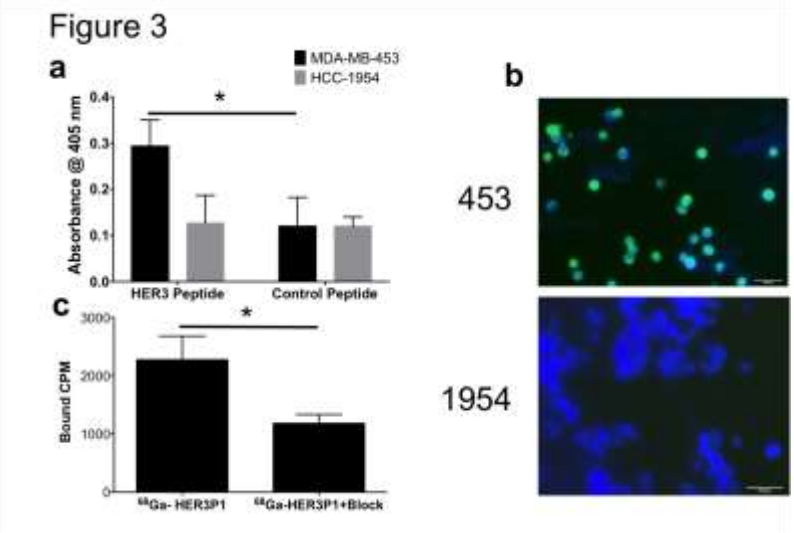
The peptide was next synthesized using standard Fmoc chemistry conjugated to a biotin for affinity and specificity determination. The affinity was calculated at 271 nM, and the peptide had a greater than 10-fold specificity in comparison to other similar proteins such as EGFR and HER2 (Figure 2). Since the peptide was confirmed to bind to HER3, the next step was to confirm binding in the cellular context for which the imaging agent would be used. As such the peptide was tested with MDA-MB-453 HER3 positive cells and HCC-1954 HER3 negative cells. Cell binding was quantified ELISA and specific binding was demonstrated to HER3+ but not HER3 – cells with the HER3P1, which was not demonstrated using a control peptide (Figure 3A).

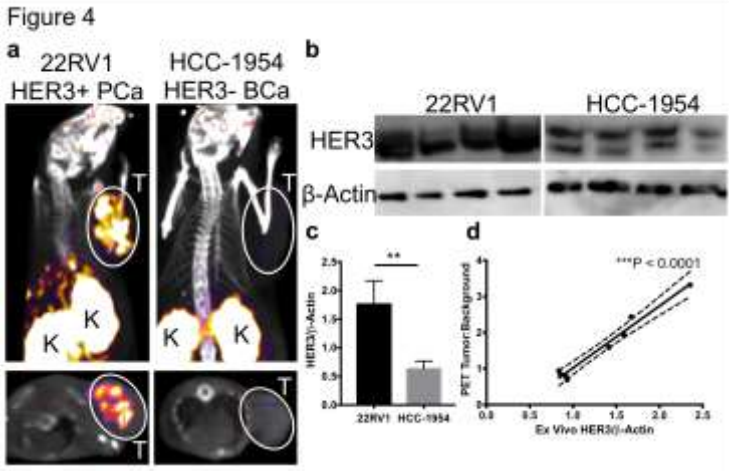
Figure 2



Furthermore, specific cellular binding was visualized using fluorescent microscopy, which confirmed specificity for HER3+ but not HER3- cells (Figure 3B). Given the excellent characteristics of the biotinylated peptide, the peptide was synthesized in the same manner, however NOTA was substituted for biotin in preparation for *in vivo* studies. The NOTA peptide was radiolabeled with ^{68}Ga and used in cell binding. The radiolabeled cell binding mimicked the fluorescent binding and confirmed that replacement of biotin with ^{68}Ga-NOTA did not alter peptide binding (Figure 3C).

The radiolabeled peptide was next prepared for *in vivo* PET imaging studies. The 22RV1 castration resistant prostate cancer cell line was chosen because it had been determined that it expressed high levels of HER3 protein suitable for PET imaging. Since all prostate cancers have HER3 expression, a breast cancer cell line was chosen as a negative control. PET imaging was performed, and very high uptake was visualized in the prostate cancer tumor, but not in the breast cancer tumor (Figure 4A). Furthermore, when the tumors were excised, protein analysis demonstrated high levels of HER3 in the prostate cancer and not in the breast cancer, consistent with PET imaging (Figure 4B-C). In fact, when the PET signal for each individual tumor was plotted against its corresponding HER3 protein level, the TBR correlated linearly with the amount of protein, indicating that the peptide was quantitatively imaging HER3 expression (Figure 4D). The peptide biodistribution was also analyzed, and the data indicated specific accumulation in tumors with minimal uptake in all normal tissues that were HER3 negative, and specific accumulation in HER3+ tissues. Additionally, the peptide was cleared renally, with relatively low levels of accumulation in comparison to other FDA-approved imaging peptides (Figure 5).





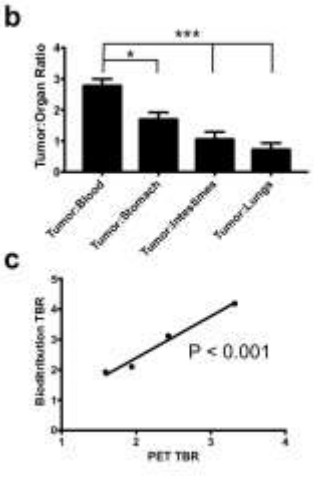
3) Significant Results or Key Outcomes

The first year of this award has resulted in significant progress towards a HER3 imaging peptide for prostate cancer. A new peptide with high affinity and sensitivity was developed. This peptide has been demonstrated to bind HER3, distinguish high HER3 from low

Figure 5

a

Organ	%ID/g
Tumor	0.50±0.18
Blood	0.18±0.04
Heart	0.31±0.07
Lungs	0.70±0.14
Stomach	0.30±0.07
Intestines	0.48±0.15
Liver	0.70±0.23
Kidney	10.1±1.67
Spleen	0.38±0.09
Muscle	0.28±0.14
Bone	0.67±0.38



HER3-expressing cells in vitro, and finally quantitatively detect levels of HER3 in vivo. These works have been presented at the annual SNMMI meeting, accepted for publication in Molecular Imaging and Biology, and have resulted in US patent application being filed for the peptide technology developed in this grant.

4) Other achievements

Based on the data generated in this project, specific funding for clinical translation of this peptide was sought and approved through an internal funding mechanism at Massachusetts General Hospital. The peptide is being prepared now for IND application, followed by IRB application and finally imaging in 10 cancer patients. The anticipated timeline for this to occur is Late 2019 or early 2020.

Major Goals (Training Specific Tasks)

Attend Harvard Catalyst Courses: I have taken and completed the Harvard Catalyst course Introduction to Translational Medicine, which was instrumental in helping to get the HER3 peptide described in this proposal funded for clinical translation.

Organize and deliver a research presentation between collaborating prostate research labs at MGH: A 2 hour seminar was organized between my mentor's (Dr. Mahmood) and Dr. Massimo Loda's lab at Dana Farber Cancer Institute to share research progress in November of 2016.

Present Research at the SNMMI, AACR or PEGS National Meetings: The work performed under this award was accepted for an oral presentation at the SNMMI annual meeting and I presented in June 2017.

Opportunities for Training and Personal Development

This award has afforded me the opportunity to present and receive feedback on my research with established prostate cancer specific and imaging specific scientists in my field. I have accomplished this through presentations with my mentor (Dr. Mahmood) collaborators (Dr. Loda), attendees of the SNMMI meeting that I presented this work, and peer-reviewers of the publication that resulted from this award. This has greatly improved my scientific direction and made me a better researcher.

Dissemination of Results to Communities of Interest

A portion of this work was presented at the SNMMI annual meeting during a time when patient advocates were invited to attend. This provided me with an opportunity to share my work with those who may be affected by my research.

Plans to Accomplish Future Goals

In order to accomplish the remaining subtasks outlined in the statement of work, the following work will be performed

Specific Aim 1- Subtask 2: While PEGylation has been tested as described above, amino acid and branched peptide structures still need to be tested. These will be synthesized by Anaspec and tested in months 14-20.

Specific Aim 2 – Subtask 1: PC-3 and MDA-PC-2b cells will be implanted and imaged using the HER3P1 peptide that has been generated in Year 1 of this proposal.

Specific Aim 2 – Subtask 2: Following imaging of a portion of the mice from Subtask 1, the tumors will be excised and subjected to SDS-PAGE and Western blots for HER3 and other signaling proteins including the androgen receptor and MAPK and PI3K/AKT pathways investigated.

4. Impact

Impact on the development of the principal disciplines of the project: The peptide developed in this application is to the best of my knowledge the first HER3 imaging peptide. This represents a significant milestone in generating imaging agents that can be used to disseminate castration resistant prostate cancer at an early stage. Furthermore, this work represents the first HER3 imaging in prostate cancer and sets an important precedent for future work corroborating HER3 with castration resistant prostate cancer.

Impact on Other Disciplines The impact of this work on the field of molecular imaging is the demonstration that HER3 is a viable imaging biomarker in prostate cancer, and it may spur further research on imaging HER3 in prostate cancer by molecular imaging scientists.

Impact on Technology Transfer The technology developed by this grant has led to US patent application being filed for the technology. Furthermore, Massachusetts General Hospital has invested $200,000 dollars in order to translate the peptide into humans in order to further investigate the efficacy of this peptide. This could in turn lead to a start-up being founded around the technology or licensing to a third-party for commercialization and wider dissemination of the product.

Impact on Society Beyond Science and Technology While in the end therapies are what are needed to cure prostate cancer, it is important to realize that characterization of these cancers is paramount to solving the problem. Cancer has been shown to a widely varied disease from patient to patient, and an increased public perception of the need for personalized medicine will help to improve care. I believe that by sharing this work as to one of the ways to characterize prostate cancer, I can help to spread the belief of investing in methods to personalize medicine and thus improve prostate cancer treatment.

5. Changes/Problems

Changes in Approach and Reasons for Change: Only slight deviations were made from the stated approach, and these did not change the fundamental approach to the project. One change was that a naïve peptide library was chosen for reselection based on characterization of the parent peptide. This resulted in a much-improved peptide sequence that was free of non-specific binding. The second minor change was to use 22RV1 cells instead of PC-3 cells, as the 22RV1 cells had much higher levels of HER3 than PC3.

Actual or anticipated problems or delays and plans to resolve them: Our PET/CT scanner was not functioning from March through July of 2017, however an alternative PET/MR has been found that will permit continuation of the project. No future problems are anticipated.

Significant changes in the use or care of human subjects, vertebrate animals, biohazards and/or select agents: Nothing to report.

6. Products

Journal Publications:

Larimer BM, Phelan N, Wehrenberg-Klee E, Mahmood U. Phage Display Selection, *In vitro* Characterization and Correlative PET Imaging of a Novel HER3 Peptide. Molecular Imaging Biology 77.9 (2017): 2318-2327. PubMed PMID: 28733706

Books or other non-periodical, one-time publications Nothing to report.

Other publications, conference papers, and presentations:
International Conference Presentation:
Larimer, Benjamin, et al. "Phage display selection of a novel HER3 PET imaging peptide for targeted therapy resistance prediction." *Journal of Nuclear Medicine* 58. supplement 1 (2017): 690-690.

Websites or Other Internet Sites: Nothing to report.

Technologies: Nothing to report.

Inventions, patent applications, and/or licenses: Novel HER3 Peptide for Imaging and Radiotherapy; US Patent Pending 62/440,052

Other Products: Nothing to report.

7. Participants and Other Collaborating Organizations

Individuals Working on the Project
Name: Benjamin Larimer
Project Role: PI
Researcher Identifier: 0000-0002-1288-7206
Nearest Person Month Worked: 7
Contribution to Project: Dr. Larimer conceived the project, performed all experiments, wrote the manuscript.
Name Umar Mahmood
Project Role: Lead Mentor
Research Identifier: NA
Nearest Person Month Worked: 0
Contribution to Project: Dr. Mahmood provided mentorship to Dr. Larimer and assisted in planning experiments and writing of the manuscript.

Changes in the Active Other Support of The PD/PI
Dr. Larimer is now an investigator on an NIH grant to Dr. Mahmood (1R01CA214744) for 1.2 calendar months.

8. Special Reporting Requirements
Nothing to report.

9. Appendices
None provided